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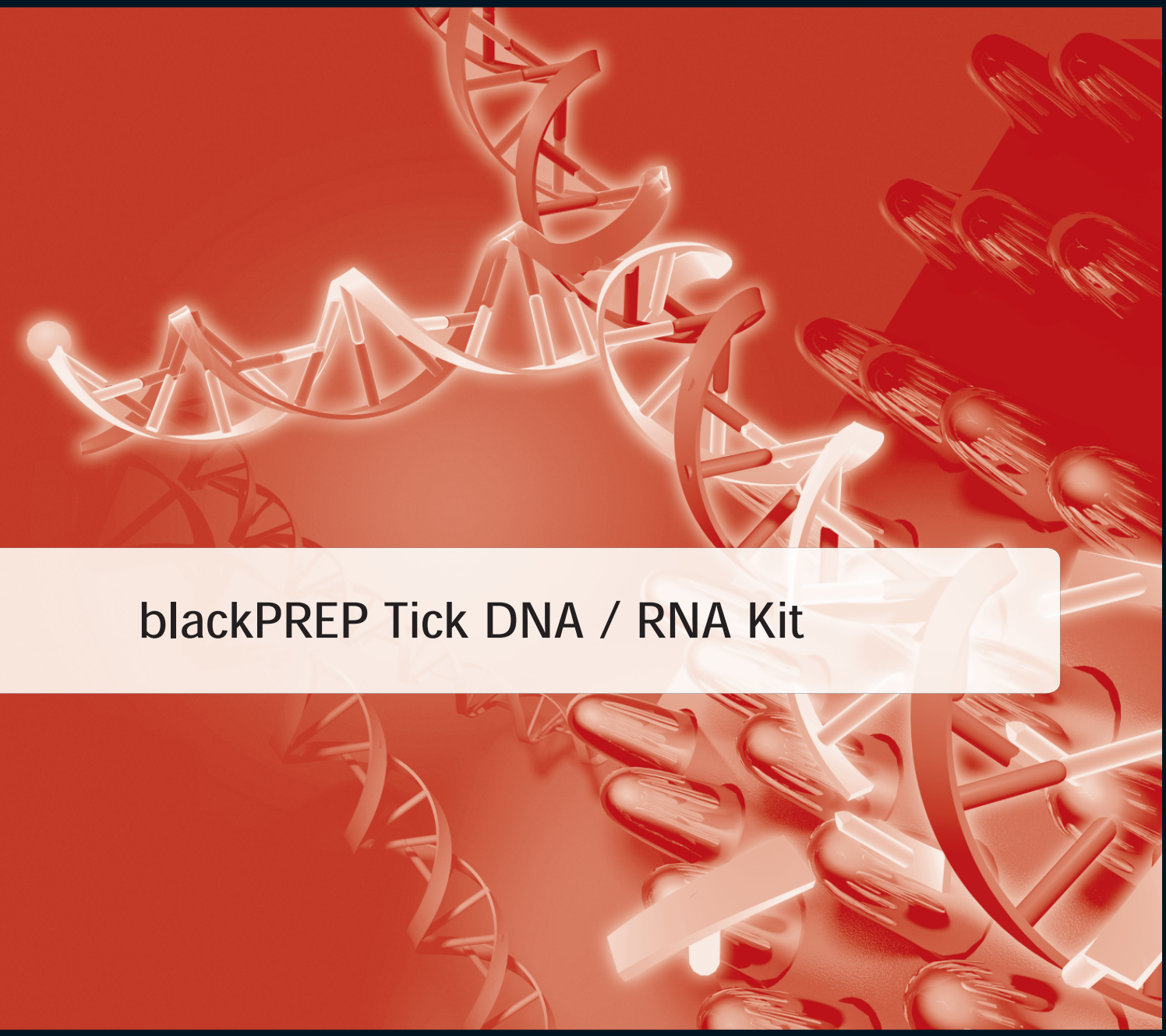
Manual



blackPREP Tick DNA / RNA Kit

Order No.:

845-BP-5100010	10 reactions
845-BP-5100025	25 reactions
845-BP-5100050	50 reactions

Publication No.: HB_KS-5100_e_110419

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Content

1 Safety precautions

All due care and attention should be exercised in handling the materials and reagents contained in the kit. Always wear gloves while handling these reagents and avoid any skin contact! In case of contact, flush eyes or skin with a large amount of water immediately.

2 Storage conditions

The blackPREP Tick DNA/RNA Kit should be stored dry, at room temperature (14 – 25 °C) and is stable for at least 12 months under these conditions. Before every use make sure that all components have room temperature. If there are any precipitates within the provided solutions solve these precipitates by careful warming.

3 Function testing and technical assistance

The Analytik Jena AG guarantees the correct function of the kit for applications as described in the manual. The components of each blackPREP Tick DNA/RNA Kit were tested by isolation of genomic DNA and total RNA from ticks and subsequent analysis on agarose gel and on Agilent Bioanalyzer.

We reserve the right to change or modify our products to enhance there performance and design. If you have any questions or problems regarding any aspects of the blackPREP Tick DNA/RNA Kit or other Analytik Jena AG products, please do not hesitate to contact us. For technical support or further information in Germany please dial +49 36 41 / 77 94 00. For other countries please contact your local distributor.

4 Product use and warranty

The user is responsible to validate the performance of the Analytik Jena AG kits for any particular use, since the performance characteristics of our kits have not been validated for any specific application. Analytik Jena AG kits may be used in clinical diagnostic laboratory systems after the laboratory has validated the complete diagnostic system as required by CLIA' 88 regulations in the U.S. or equivalents in other countries.

All products sold by Analytik Jena AG are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately.

Note

For research use only!

5 Kit components

Important!
Kit components are stored at room temperature.

	10 Extractions	25 Extractions	50 Extractions
Lysis Tube P	10	25	50
Lysis Solution RL	6 ml	12 ml	25 ml
Washing Solution HS	6 ml (final volume 12 ml)	15 ml (final volume 30 ml)	30 ml (final volume 60 ml)
Washing Solution LS	3 ml (final volume 15 ml)	8 ml (final volume 40 ml)	16 ml (final volume 80 ml)
Elution Buffer	2 ml	5 ml	10 ml
RNase-free water	2 ml	2 x 2 ml	5 ml
Spin Filter D	10	25	50
Spin Filter R	10	25	50
Receiver Tubes (2.0 ml)	2 x 40	4 x 50	8 x 50
Elution Tubes (1.5 ml)	20	50	2 x 50
Manual	1	1	1
Initial steps	• Add 6 ml of 96-99.8 % ethanol to the bottle Washing Solution HS, mix thoroughly and keep the bottle always firmly closed! • Add 12 ml of 96-99.8 % ethanol to the bottle Washing Solution LS, mix thoroughly and keep the bottle always firmly closed!	• Add 15 ml of 96-99.8 % ethanol to the bottle Washing Solution HS, mix thoroughly and keep the bottle always firmly closed! • Add 32 ml of 96-99.8 % ethanol to the bottle Washing Solution LS, mix thoroughly and keep the bottle always firmly closed!	• Add 30 ml of 96-99.8 % ethanol to the bottle Washing Solution HS, mix thoroughly and keep the bottle always firmly closed! • Add 64 ml of 96-99.8 % ethanol to the bottle Washing Solution LS, mix thoroughly and keep the bottle always firmly closed!

6 Recommended steps before starting

- Ensure that the Washing Solution HS and Washing Solution LS have been prepared according to the instruction (→ "Kit components", p. 4)
- Centrifugation steps should be performed at room temperature
- Avoid freezing and thawing of starting materials

7 Components not included in the kit

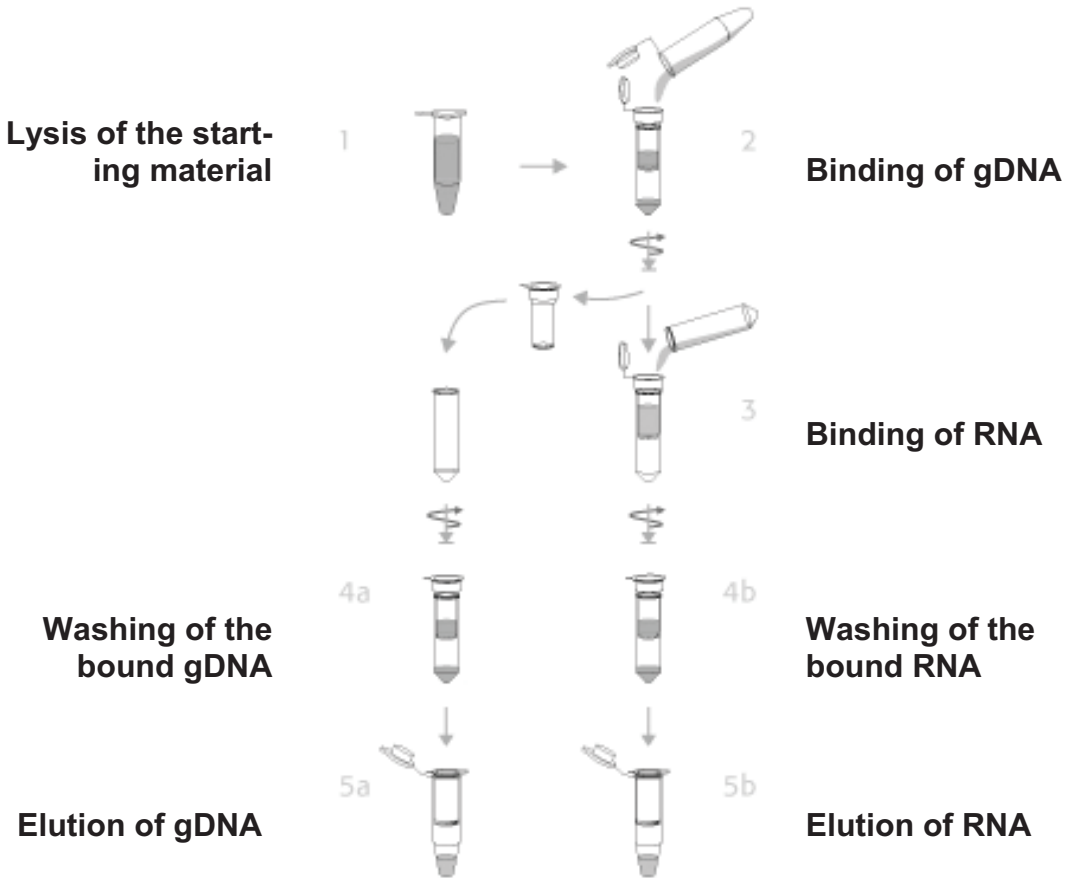
- DNase I; optional
- Lysozym; optional
- ddH_2O
- TE-Buffer (10 mM Tris-HCl; 1 mM EDTA; pH 8,0); optional
- Reaction tubes
- Ethanol (70 %, 96 – 99,8 %)

8 Parallel Extraction of DNA and RNA

8.1 Product description

The blackPREP Tick DNA/RNA Kit offers the simultaneous isolation of DNA and RNA directly from ticks. Especially in case of determination of e.g. RNA viruses (e.g. determination of FSME) beside the detection of bacterial pathogens, this kit will be useful.

8.2 General procedure for nucleic acid extraction



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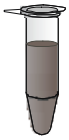
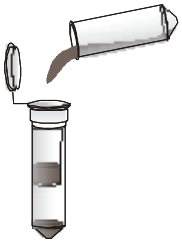
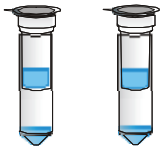
blackPREP Tick DNA / RNA Kit

Protocol: Isolation of DNA and RNA from ticks

Recommended steps before starting

- Prepare Washing Solution HS and Washing Solution LS according to the instruction

1.	Starting material			▪ Whole ticks
2.	Homogenization and lysis	▪ Homogenizer e.g. SpeedMill		▪ Add the tick into a Lysis Tube P ▪ Add 100 µl RL ▪ Homogenization: 4 min
				▪ Add 300 µl RL ▪ Incubation: 30 min @ RT ▪ Centrifugation: max. speed
3.	Binding of DNA New Receiver Tubes			▪ Spin Filter D to Receiver Tube ▪ Add supernatant to Spin Filter D ▪ 10.000 x g (12.000 rpm): 2 min ▪ Spin Filter D to Receiver Tube ▪ **Don't** discard the filtrate!
4.	Binding of RNA New Receiver Tubes			▪ Add 350 µl 70 % ethanol to filtrate from step 3 ▪ Spin Filter R to Receiver Tube ▪ Add filtrate to Spin Filter R ▪ 10.000 x g (12.000 rpm): 2 min ▪ Spin Filter R to Receiver Tube
5.	Washing of Spin Filter D and R New Receiver Tubes			▪ Add 500 µl HS to each ▪ 10.000 x g (12.000 rpm): 1 min ▪ Add 650 µl LS to each ▪ 10.000 x g (12.000 rpm): 1 min

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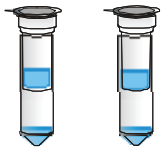
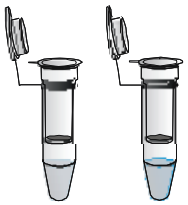
6. Remove Ethanol of Spin Filter D and R

New Receiver Tubes



- Discard filtrate
- Spin Filter D to Receiver Tube
- Spin Filter R to Receiver Tube
- Centrifuge: max speed, 2 min

7. Elution of Spin Filter D and R



- Spin Filter D to an Elution Tube
- Add 100 µl Elution Buffer
- Spin Filter R to an Elution Tube
- Add 30-80 µl RNase-free water
- Incubation: 1 min @ RT
- 6.000 x g (8.000 rpm): 1 min

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This documentation describes the state at the time of publishing. It needs not necessarily agree with future versions. Subject to change!

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9 General notes and safety recommendations on handling RNA

RNA is far less stable than DNA. It is very sensitive to degradation by endogenous RNases in the biological material and exogenous RNases which are permanently present everywhere in the lab. To achieve satisfactory qualitative and quantitative results in RNA preparations, contaminations with exogenous RNases have to be reduced to a minimum in accordance with the following recommendations:

- Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contaminations from surface of the skin or from dusty laboratory equipment.
- Change gloves frequently and keep tubes closed.
- Keep isolated RNA on ice.
- Reduce preparation time as much as possible.
- Use only sterile, disposable polypropylene tubes throughout the procedure (these tubes are generally RNase-free.)
- Non-disposable plastic ware should be treated before use to ensure that it is RNase-free. Plastic ware should be thoroughly rinsed with 0.1 M NaOH, 1 mM EDTA followed by RNase-free water. You can also take chloroform-resistant plastic ware rinsed with chloroform to inactivate RNases.
- All glassware should be treated before use to ensure that it is RNase-free. Glassware should be cleaned with detergent, thoroughly rinsed and oven baked at 240 °C for four or more hours before use. Autoclaving alone will not inactivate many RNases completely. Oven baking inactivates RNases and ensures that no other nucleic acids (such as plasmid DNA) are present on the surface of the glassware. You can also clean glassware with 0.1 % DEPC (diethyl pyrocarbonate). The glassware has to be immersed in 0.1 % DEPC solution for 12 hours at 37 °C and then it has to be autoclaved or heated to 100 °C for 15 min to remove residual DEPC.
- Electrophoresis tanks should be cleaned with detergent solution (e.g. 0.5 % SDS), thoroughly rinsed with RNase-free water, rinsed with ethanol and finally allowed to dry.
- All buffers have to be prepared with DEPC-treated RNase-free ddH_2O.
- Avoid handling bacterial cultures, cell cultures or other biological sources of RNases in the same lab where the RNA purification will be performed.
- Do not use equipment, glassware and plastic ware employed for other applications which might introduce RNase contaminations in the RNA isolation.

10 Protocol: DNA and RNA extraction form ticks

A. Homogenizatio of the tick using SpeedMill or another homogenizer

Note

To maximize the final yield of DNA and total RNA a complete homogenization of tick is important!

1. Transfer the whole tick into a Lysis Tube P and add **100 µl Lysis Solution RL**. Close the Lysis Tube P firmly.
2. Place the Lysis Tube P in the sample holder of the SpeedMill as described in the user manual of the device.
3. Homogenization: 4 min

   Note: If the tick is not homogenized completely, continue the homogenization process. In case of using another homogenizer based on beads, please follow the recommendations of the manufacturer!
4. Add **300 µl Lysis Solution RL** to the homogenized tick and incubate the sample under continuous shaking for 30 min at room temperature

B. Binding of DNA onto Spin Filter D

1. After incubation of the sample centrifuge the Lysis Tube P at maximum speed to spin down unlysed material. Place a Spin Filter D into a 2.0 ml Receiver Tube. Transfer the supernatant of the lysed sample onto the Spin Filter D. Centrifuge at 10.000 x g (~12.000 rpm) for 2 minutes.

   Do not discard the filtrate, because the filtrate contains the RNA!

   Note: If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.
2. Place the Spin Filter D into a new 2.0 ml Receiver Tube. The DNA is bound onto Spin Filter D. Processing of the Spin Filter D will be continued after binding of total RNA onto Spin Filter R (→ chapter D).

C. Binding of RNA onto Spin Filter R

1. Place a Spin Filter R into a new 2.0 ml Receiver Tube. Add **350 µl of 70 % ethanol** to the filtrate from step B.1. Mix the sample by pipetting up and down several times. Transfer the sample onto the Spin Filter R. Centrifuge at 10.000 x g (~12.000 rpm) for 2 minutes.

   Note: If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

2. Discard the 2.0 ml Receiver Tube with filtrate and place the Spin Filter R into a new 2.0 ml Receiver Tube. The total RNA is bound on Spin Filter R. Both Spin Filters (Spin Filter D and R) will be processed in parallel now.

D. Parallel processing of both – Spin Filter D for isolation of DNA and Spin Filter R for isolation of RNA

1. Open the Spin Filters D and R, add **500 µl Washing Solution HS** to each, close the caps and centrifuge at 10.000 x g (~12.000 rpm) for 1 minute. Discard the Receiver Tubes with the filtrate and place both Spin Filters D and R into new Receiver Tubes
2. Open the Spin Filters D and R, add **650 µl Washing Solution LS** to each, close the caps and centrifuge at 10.000 x g (~12.000 rpm) for 1 minute. Discard the Receiver Tubes with the filtrate and place both Spin Filters D and R into new Receiver Tubes
3. Centrifuge at 10.000 x g (~12.000 rpm) for 2 minutes to remove all traces of ethanol. Discard the 2.0 ml Receiver Tubes.
4. Place the Spin Filters D (DNA elution) and Spin Filter R (RNA elution) each into a 1.5 ml Elution Tube. Carefully open the caps of the Spin Filters D and R, add **100 µl Elution Buffer** to Spin Filter D and **30-80 µl RNase-free water** to Spin Filter R. Incubate at room temperature for 2 minutes. Centrifuge at 5.000 x g (~6.000 rpm) for 1 minute.

Note

Depending on the extracted yield or the needed concentration of DNA or total RNA it is also possible to elute with different volumes of Elution Buffer/RNase-free water. A lower volume of Elution Buffer/RNase-free water increases the concentration of DNA/RNA and a higher volume of Elution Buffer/RNase-free water leads to an increased yield but a lower concentration of nucleic acids. Please note, that the minimum of RNase-free water should be 20 µl.

Store nucleic acids at appropriate conditions (RNA at –80 °C and DNA at –20 °C)!

11 Troubleshooting

Problem / probable cause	Comments and suggestions
Clogged Spin Filter	
• Insufficient disruption or homogenization	After lysis centrifuge lysate to pellet debris and continue with the protocol using the supernatant. Reduce amount of starting material.
Little or no DNA or total RNA eluted	
• Insufficient disruption or homogenization	Reduce amount of starting material. Overloading reduces yield!
• Incomplete elution	Prolong the incubation time with Elution Buffer and RNase-free water to 5 minutes or repeat elution step once again.
DNA contamination of extracted RNA	
• Too much starting material	Reduce amount of starting material.
• Incorrect lysis of starting material	Use the recommended techniques for lysis of cell pellet. Perform DNase digest of the eluate containing the total RNA or perform a on column DNase digest step after binding of the RNA on Spin Filter R!
Total RNA degraded	
• RNA source inappropriately handled or stored	Ensure that the starting material is fresh! Ensure that the protocol, especially the first steps, has been performed quickly.
• RNase contamination of solutions; Receiver Tubes, etc.	Use sterile, RNase-free filter tips. Before every preparation clean up the pipette, the devices and the working place. Always wear gloves!
Total RNA does not perform well in downstream applications (e.g. RT-PCR)	
• Ethanol carryover during elution	Increase time for removing of ethanol.
• Salt carryover during elution	Ensure that Washing Solution HS and Washing Solution LS are at room temperature. Check up Washing Solution for salt precipitates. If there are any precipitate dissolves these precipitate by carefully warming.

engl. 04/11 – Analytik Jena AG, Jena

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